Title: **Protein Assay Protocol**

Author:	Pete Key	Date:
Program Manager:	Michael H. Fulton	Date:
Branch Chief:	Geoffrey I. Scott	Date:

1.0 OBJECTIVE

To conduct protein assay.

2.0 HEALTH AND SAFETY

Personnel should wear a lab coat and chemical resistant gloves. Personnel should be aware that a phenolic compound is used in this assay and due caution should be taken.
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3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Any employee who routinely works in the laboratory should be capable of performing this task. Training of new staff should be carried out under supervision of an experienced technical employee familiar with this SOP before the employee can work unsupervised.

4.0 REQUIRED AND RECOMMENDED MATERIALS

This section lists the required supplies and equipment:

spectrophotometer	cuvettes
assay reagents,buffers,standard	pipetors
lab coat	test tubes
chemical resistant gloves	

5.0 PROCEDURE

5.1 Protein Assay

1. Make Lowry Reagent, Folin & Ciocalteu's Phenol Reagent and Protein Standard according to **Protein Assay Notebook** in Room 230.

2. Prepare standards and blank for 50 µl and 100 µl protein samples according to Table in Protein Assay Notebook in Room 230.

3. Prepare Samples {See **Protein Assay Notebook** in Room 230}

   a.) 50 µl samples: Dilute samples with 950 µl D.I. water to acquire 1.0 ml total sample volume
   100 µl samples: Dilute samples with 900 µl D.I. water to acquire 1.0 ml total sample volume

   b.) Add 1.0 ml Lowry Reagent to ALL tubes (Blanks, Standards, Samples).
   Do not start mixing until **last** tube is filled.
   Then vortex, then let stand for 20 minutes at room temperature.

   c.) Add 500 µl of Folin & Ciocalteu's Phenol Reagent to ALL test tubes and vortex after each addition.
   Start 30 minute countdown for each one after each has been mixed.

   d.) Transfer solutions to cuvettes and read in spectrophotometer at 500 nm.

4. Discard all reagents, standards and samples with plenty of water after use.

5.2 Spectrophotometer Setup for Protein Assay

1. Turn on Spec unit, then module, then printer

2. Key in "500", Press "2nd" then "Go to λ"

3. Insert Blank - Pour blank into cuvette and place into spectrophotometer

4. Press "2nd" then "Zero"

5. After zeroing, set for Standard Mode
   Press "5" then "2nd" then "Select"

SAVE TESTS	"no"
STDS	"yes"
THRU 0	"no"
# STDS	Enter number of standards then Press "yes"

6. Insert Standards. Remove blank and pour standard into cuvette

7. Press "yes" or "send"

8. Enter concentration of Standard then "yes" or "send".
   Repeat steps 6 and 7 until all Standards have been entered.

a. Check printout for slope value. Should 1.00 or very close to 1.00.

Keep blank and standard cuvettes in order.

9. ACCEPT NAME	Press "yes" to accept Press "no" to change letters. Use "yes" and "no" to move between letters. Press "Send" to choose letters; "Stop" when finished.
10. ID #	Press "yes" (to change ID see step 9)

11. WV=500	"yes"
INIT DELAY	"0" then "Send"
LO	"yes"
HI	"yes"
SLOPE	"yes"
INTERCEPT	"yes"
SIG AVG	"yes"

12. Insert each sample into spec chamber and press "Run" on the module.

13. Check protein readout on printout. If numbers are greater than 10% or less than 10% of the two standards used, repeat steps 3 – 12 with standards that bracket the sample readout.

6.0 QUALITY CONTROL/QUALITY ASSURANCE

Personnel should adhere to good laboratory practices performing this assay. This procedure should always be performed with proper precautions to minimize personnel exposure to reagents.

7.0 REFERENCES

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